Methyltetrahydrofolate reductase (MTHFR) Mutations in Healthy Individuals in Ninavah Province, Iraq

Sura O. AL-Dewachi¹, Muna A. Kashmoola²

¹Department of Pathology, Ninevah Medical College- Ninevah University, Iraq
²Department of Pathology, Mosul College of Medicine, University of Mosul, Iraq

Corresponding author:
Sura O. AL-Dewachi
suraaldewachi@yahoo.com

Abstract
Methyltetrahydrofolate reductase (MTHFR) is an enzyme encoded by the MTHFR gene, it plays an important role in homocysteine metabolism, and so genetic mutations of this enzyme cause a reduction in enzymatic activity and hyperhomocysteinemia. One of the most common MTHFR gene mutations is C677T. This study is aimed to determine the frequency distribution of (C677T) MTHFR mutation in healthy subjects from Ninavah Province -Iraq. The sample of this study includes 150 randomly selected apparently healthy subjects who are attending pre-marriage screening center in Mosul for routine pre-marriage checking. DNA was isolated from the blood samples of all subjects and investigation for MTHFR (C677T) gene polymorphism was done by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) of a genomic DNA fragment at nucleotide 677. The most frequently observed MTHFR genotype was Wild CC 65.3%, followed by Heterozygous CT 28.0%, and, Homozygous TT had the lowest frequency of 6.7%. No significant association was found between genotype and sex. In conclusion, we have defined the frequency distribution of (C677T) MTHFR gene mutation in the healthy subjects from Ninavah Province/ Iraq. These results could be of help in genome association studies and in the clinical encounter.

Keywords: Methyltetrahydrofolate reductase (MTHFR) gene, polymorphism, C677T, PCR, RFLP

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Introduction
Methyltetrahydrofolate reductase is a rate-limiting enzyme in the methyl cycle and is encoded by the MTHFR gene. It is involved in folate metabolism and catalyzes the conversion of 5, 10- methyl-THF to 5 methyl-THF; a cofactor of homocysteine methylation, that results in the generation of methionine. The reduction in function or amount of (MTHFR) leads to hyperhomocysteinemia (1). Two common genetic polymorphisms in the MTHFR gene have been recognized; C677T and A1298C single-nucleotide polymorphisms (2, 3). In C677T single nucleotide polymorphism cytidine residue at position 677 in the gene is substituted by thymidine causing an alanine-to-valine conversion in the protein. The resultant variant is thermolabile; so the enzyme activity is reduced by 35% in carrier’s genotype C/T, in comparison to normal genotype C/C, while it’s reduced by 70% in homozygous genotype T/T (2,3).

The other single nucleotide polymorphism, A1298C, which is located within the enzyme regulatory domain, results in glutamate to alanine substitution and reduces MTHFR enzyme activity in a mild form (4,6). It has been reported that there is association between Genetic variations of MTHFR and the gene deficiency and this may influence individual susceptibility to occlusive vascular disease including thrombosis and atherosclerosis, neural tube defects, mental disorders (depression and schizophrenia), neurodegenerative disorders (Alzheimer’s disease and Parkinson’s disease), and cancers (colon cancer, prostate, and breast cancer) (7-13). There are also suggests that decreased MTHFR activity caused by 677C>T polymorphism in mothers may increase the risk of Down syndrome in their children (14, 15). Among Asians, the gene polymorphism was studied in Japanese and Sri Lankans only (16). Limited data are available for some of the Middle East countries especially Jordan and Saudi Arabia, and no data available

for Iraq (17, 18). This study is aiming to determine the frequency distribution of (C677T) MTHFR mutation in healthy subjects from Ninavah Province-Iraq.

Materials and method
The sample of this study includes 150 randomly selected apparently healthy subjects who were attending premarital screening centers in Mosul for the period between the 1st of October 2017 and 30th of March 2018 for a routine mandatory premarital checkup. Medical checking was done for all participants before involvement in the study and recruited on the basis of the absence of any known diseases. Some attention was given to the absence of reported malignancy; of cardiovascular, kidney, liver, or genetic disorders; and of vitamin supplementation. All participants were informed about the study protocol and written consent was taken from all of them. The study was approved by the local Medical Ethics Committee of Ninavah Directorate of Health in Mosul. From each participant, 1 mL of whole blood was collected in EDTA tube for isolation of genomic DNA using a kit from Promega. According to Frosst et al, PCR-RFLP analysis was used to determine the (C677T) MTHFR gene mutation by using Hinf I restriction analysis of a 198-bp polymerase chain reaction–amplified fragment in the gene for MTHFR (19).

PCR protocol for MTHFR 677
Initial- Denaturation-94°C for 8 Min, Denaturation:94°C for 1 min, Annealing:63°C for 1 min, Extension: 72°C for 1 min, Final extension at 72°C for 7 min, repeated for 40 cycles using applied biosystem. Then amplified PCR products (MTHFR) were subjected to HinfI restriction enzyme for digestion at 37°C in a water bath overnight. Digestion was carried out in a final volume of 10µL, using 8.5µL of PCR product, 5 units of HinfI enzyme, and 1.0µL of the buffer. Then an analysis of the restriction fragments was done by gel electrophoreses of digested PCR products using 3% agarose and stained with ethidium bromide (20). The sequence of the restriction enzyme HinfI is recognized by the help of Polymorphism C677T, and this is detected by digestion of the 198-bp PCR product, generating 23- and 175-bp fragments for the polymorphism in homozygosis genotype TT. The presence of a 198-bp fragment is identified as (genotype CC), and the manifestation of three fragments, 198 bp, 175bp, and 23 bp is represented as genotype CT, while two fragments, 175 and 23 bp presence is recognized as genotype TT (21).

Statistical analysis
Frequency analysis was used to describe the categorical variables (gender and genotype). Association between variables was tested using the Chi-square test. Statistical analysis was performed by using SPSS statistical program for V23.

Results:
The study included 150 individuals, 75 (50%) males, and 75 (50%) females; their age ranges 20-50 years for males and 16-41 years for females. The amplification using primers on DNA extracted on the study sample revealed a 198 bp product, with restriction digestion of amplified products with Hinf I produced a 175 with 198 bp fragments for heterozygous (CT genotype), and complete digestion resulting in 175 bp and faint 23 bp was too faint fragments for homozygous (TT genotype). Undigested product length of 198 kb was labeled as Wild genotype (CC). Figure (1). The most frequently observed genotype was Wild CC (98 individual, 65.3%), followed by Heterozygous CT 42 (28.0%), and Homozygous TT showed the least frequency (10 individuals, 6.7%). Figure (2) No statistically significant difference was found between different MTHFR genotypes and sex, p value= 0.229. Table (1)
Figure (1): PCR analysis of MTHFR genotypes in healthy individuals from Ninavah Province -Iraq.

Figure (2): The percentage of MTHFR C677T genotype mutations in healthy individuals from Ninavah Province -Iraq.

Table (1): The frequency MTHFR C677T genotype mutations in healthy individuals from Ninavah Province –Iraq.

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th>Malen=75n (%)</th>
<th>Femalesn=75n (%)</th>
<th>Total n=150</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous TT</td>
<td>6 (60.0)</td>
<td>4(40.0)</td>
<td>10</td>
<td>0.229</td>
</tr>
<tr>
<td>Heterozygous CT</td>
<td>25(59.5)</td>
<td>17(40.5)</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Wild CC</td>
<td>44(44.9)</td>
<td>54(55.1)</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

*Using chi square test
Discussion:
Functional polymorphisms of the genes encoding enzymes that are involved in metabolism may lead to increase susceptibility to a variety of diseases, therefore, (MTHFR) gene polymorphisms are one of the most considered (22). The critical protein in the methylation of homocysteine in the MTHFR enzyme. Among dozen polymorphisms of the MTHFR gene, two common variants are described: A1298C and C677T (4, 5). Results of studies regarding the frequency of MTHFR C677T polymorphism worldwide are quite diverse and mostly focus on 677TT genotype because of clear connection with reduced MTHFR activity is related to this polymorphic variant. The homozygous 677TT genotype has a reduced enzyme activity by 30% with a prevalence ranging from 8 to 10% of the population. However, Heterozygotes have reduced enzyme activity by nearly 60% with a prevalence of 40% of the general population (23, 24).

There are also remarkable differences as to the frequency of MTHFR 677T polymorphism within populations of one race, inhabitants of one continent, country or region. (23-28). Up to our knowledge, this research is the first that investigated the frequency distribution of the C677T MTHFR gene mutation in healthy individuals in Iraq. Our findings revealed that the frequency of 677TT genotype among Iraqis is 6.7%, which is a relatively low rate. These results are comparable to that of other studies done on Turkish population(9.6%) (29), Jordanian (8%) (30), Lebanese (3.9%) (31), Bahraini (2.63%) (32), and Moroccan (5.98%) (33). However, the higher rate of 677TT genotype was reported in studies done on Mexican (35.7%) (34), Chinese (32.2%) (35), Italian (26.4%) (26), and on Costa Rican Amerindian (70.07%) (36).

In the present study carriers of both sexes show no significant differences in the prevalence of C677T MTHFR polymorphism. Most of the researches revised did not postulate the sex arrangement of the samples, did not explain differences in genotype frequencies by gender, or described that genotype frequency was not significantly different in both sexes (24, 37). In conclusion, we have defined the frequency distribution of (C677T) MTHFR gene polymorphism in healthy persons from Ninavah Province-Iraq. These results might be beneficial in genome-wide association studies and in clinical testing settings. The information of like results in the Middle East area through population-based studies will donate to a better understanding of the interaction of genetic and environmental risk factors fundamental certain disease.

References


